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Incubation experiments using nitrogen isotope discrimination to estimate ammonia emission from amended sheep manure treatments

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Animal Research Paper

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Abstract

Two 10-day *in vitro* experiments were conducted to investigate the relationship between nitrogen (N) isotope discrimination (δ15N) and ammonia (NH_3) emissions from sheep manure. In Exp. 1, three different manure mixtures were set up: control (C); C mixed with lignite (C + L); and grape marc (GM), with 5, 4 and 5 replications, respectively. For C, urine and faeces were collected from sheep fed a diet of 550 g lucerne hay/kg, 400 g barley grain/kg and 50 g faba bean/kg; for C + L, urine and faeces were collected from sheep fed the C diet and 100 g ground lignite added to each incubation system at the start of the experiment; for GM, urine and faeces were collected from sheep fed a diet consisting of C diet with 200 g/kg of the diet replaced with GM. In Exp. 2, three different urine-faeces mixtures were set up: 2U:1F, 1.4U:1F and 1U:1F with urine to faeces ratios of 2:1, 1.4:1 and 1:1, respectively, each with 5 replications. Lignite in C + L led to significantly lower cumulative manure-N loss by 81 and 68% in comparison with C and GM groups, respectively ($P = 0.001$). Cumulative emitted manure NH_3-N was lower in C + L than C and GM groups by 35 and 36%, respectively ($P = 0.020$). Emitted manure NH_3-N was higher in 2U:1F compared to 1.4U:1F and 1U:1F by 18 and 26%, respectively ($P < 0.001$). This confirms the relationship between manure δ15N and cumulative NH_3-N loss reported by earlier studies, which may be useful for estimating NH_3 losses.

Introduction

Ammonia (NH_3) emission from livestock manure has major negative effects on the environment including causing acid rain, eutrophication of surface waters, fine particulate matter formation in the air (Hristov *et al.*, 2011), and respiratory diseases in humans (Hristov *et al.*, 2011). Emitted NH_3 can be converted to nitrous oxide (N_2O), a greenhouse gas (GHG), which contributes to global warming (Chen *et al.*, 2015). Therefore, there is an urgent need to assess and mitigate NH_3 emissions from livestock manure globally.

Previous studies showed that feed manipulations, such as the inclusion of grape marc (GM; i.e., grape pomace) can reduce livestock urinary nitrogen (N) (UN):faecal N (FN) ratio (UN:FN) in dairy cattle (Greenwood *et al.*, 2012; Wu *et al.*, 2022) and this may reduce NH_3 emission from manure (Lynch *et al.*, 2007). It is known that GM has a moderate to high tannin and fat content (Spanghero *et al.*, 2009) and this can increase the ruminal undegradable protein supply, resulting in a shift in the site of N excretion from urine to faeces. Faecal N is more stable and less readily converted to NH_3 than UN. Chen *et al.* (2015) and Sun *et al.* (2016) demonstrated that application of 3 to 6 kg/m^2 lignite to a feedlot cattle pen surface can reduce manure NH_3 loss by 30 to 66%. Lignite has three major chemical characteristics that may be related to the reduction of manure NH_3 emission: (1) low pH (3.69), (2) high cation exchange capacity (CEC; 96.8 centimole (+)/kg, and 3) high labile carbon content of up to 200 g/kg (Husted *et al.*, 1991; McCrory and Hobbs, 2001; Chen *et al.*, 2015). These chemical and physical characteristics limit the conversion of manure-N into NH_3 and leaves NH_3 in the NH_4^+ form that is not emitted.

One of the major challenges to manage NH_3 emissions from livestock manure is the availability of accurate and practical methods to estimate emissions. Previous reviews clearly demonstrated the direct methods to accurately quantify NH_3 emission (e.g., micrometeorological methods), but they are heavily influenced by many environmental factors, such as temperature and wind speed, and they are often costly and labour intensive in large scale operation (Hristov *et al.*, 2011). Therefore, the research focus has shifted to explore indirect methods to quantify NH_3 emission from manure. This includes biomarkers such as manure-N to potassium (K) ratio and N isotopic discrimination (δ^{15}N (‰) = [(^{15}N/^{14}N) sample – (^{15}N/^{14}N) air]/[^{15}N/^{14}N] air × 1000) (Hristov *et al.*, 2009; Lee *et al.*, 2011). Due to physical isotopic

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discrimination, NH_3 emitted from manure is highly depleted in ^{15}N, which resulted in manure becoming progressively enriched in ^{15}N over time. This change in manure ^{15}N was useful to describe cumulative NH_3 emissions from dairy cow manure over a 15-day *in vitro* incubation (Hristov *et al.*, 2009). However, a follow up study from the same group showed that the positive relationship was only sustained for the first 6 days of *in vitro* incubation (Lee *et al.*, 2011). The reason for the discrepancy is not clear, but it may be related to different manure properties (Tamminga, 1996) or the incubation environment (Ndegwa *et al.*, 2008; Hristov *et al.*, 2009). Further research is needed to confirm when and how $\delta^{15}N$ can be used to predict NH_3 emissions from livestock manure.

To the best of authors' knowledge, no study has so far explored the relationship between $\delta^{15}N$ and emitted NH_3 with sheep manure. Therefore, this study aimed to use lignite application and GM feeding to induce changes in sheep manure NH_3 emissions and investigate the relationship between $\delta^{15}N$ and emitted manure NH_3 in sheep manure over a 10-day *in vitro* incubation. Also, the study aimed to investigate how different ratios of sheep urine to faeces can affect NH_3 emissions and the relationship between $\delta^{15}N$ and emitted manure NH_3 in sheep manure over a 10-day *in vitro* incubation. The authors hypothesized that the use of lignite application in sheep manure and the inclusion of GM into sheep diets can significantly reduce manure NH_3 emissions.

Materials and methods

Manure preparation in experiment 1

The background experiment was described by Wu *et al.* (2022) and the summary dietary information for the current *in vitro* experiment is presented in Table 1. Four sheep were used as donors for urine and faeces. Total excreted urine and faeces were measured and collected from two sheep fed with control (C) and two sheep fed GM diet, and no preservative was used for urine and faeces collections (the sheep were adapted for a period of 14 days on each diet and then, the urine and faeces were collected in a period of 6 days). To reduce NH_3 emissions from excreted urine, the temperature of the sheep urine was kept below 10°C, by cooling excreted urine with ice blocks during the collection process. The collected urine and faeces were kept at −20°C prior to analysis. Fourteen incubation systems were set up, with three different manure mixtures (Table 1): C with 5 replications; C mixed with lignite (C + L) with 4 replications; and GM with 5 replications. For C, urine and faeces were collected from sheep fed a diet of 550 g lucerne hay/kg, 400 g/kg barley grain, and 50 g bean/kg; for C + L, urine and faeces were collected from sheep fed the C diet and 100 g ground lignite was added to each incubation system at the start of the experiment; and for GM, urine and faeces were collected from sheep fed the C diet with 200 g/kg of C diet replaced with GM (fresh matter basis). Faeces samples were removed from the freezer and processed twice using a juicer (Breville BJE410CRO The Juice Fountain Max juicer, Breville, China) to break up faecal particles; faeces were then re-frozen prior to reconstruction with urine to form a manure mixture. Urine and faeces were thawed and immediately reconstructed using a blender (300-W of 600-ml electric portable mini blender with a glass jar, ANKO Ltd, China) based on the urine to faeces volume ratio excreted by the animals for each dietary treatment. A porcelain pestle

Table 1. The design and input material for *in vitro* Experiment 1 and Experiment 2

Exp.	Treatment	Rep.	Urine (g)	Faeces (g)	Urine/ faeces (g/g)	Manure (g)	Lignite (g)	Manure mixture (g)	UN (g)	FN (g)	UN /FN (g/g)	Manure-N (g)	Manure-N + lignite-N (g)
1	C	5	300	162	1.85	462	0	462	1.06	1.29	0.82	2.35	2.35
	C + L	4	300	162	1.85	462	100	562	1.06	1.29	0.82	2.43	2.98
	GM	5	300	234	1.28	534	0	534	1.02	1.78	0.58	2.80	2.80
2	2U:1F	5	400	200	2	600	–	600	1.76	1.15	1.52	2.91	2.91
	1.4U:1F	5	350	250	1.4	600	–	600	1.54	1.44	1.07	2.98	2.98
	1U:1F	5	300	300	1	600	–	600	1.32	1.73	0.76	3.05	3.05

C, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg); C + L, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg) and mixed with 100 g lignite; GM, urine and faeces from sheep fed grape marc diet (control animal feed ration, 200 g/kg replaced with grape marc);
2U:1F, ratio of urine to faeces = 2:1; 1.4U:1F, ratio of urine to faeces = 1.4:1; 1U:1F, ratio of urine to faeces = 1:1; UN, urinary nitrogen; FN, faecal nitrogen.
All presented units are based on each incubation system.

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and mortar (115 mm/4½" diameter, weight 820 g, and glazed finish) was used to grind the lignite. Then, lignite was passed through a 500 µm mesh size sieve.

Manure preparation in experiment 2

The design and input material for Exp. 2 are presented in Table 1. Six sheep were used as donors of urine and faeces. Urine and faeces samples were collected without preservatives. The collected urine and faeces were kept in a freezer at −20°C prior to analysis. Faeces samples were processed twice using a domestic blender to break up faecal particles; faeces were then re-frozen prior to reconstruction with urine to form a manure mixture. Urine and faeces were thawed and immediately reconstructed using a blender (300-W of 600-ml electric portable mini blender with a glass jar, ANKO Ltd, China) based on the urine to faeces volume ratio excreted by the animals per dietary treatments. Fifteen incubation systems were set up, with replicates of three different volumes of urine-faeces mixture (Table 1): (1) 2U:1F (urine to faeces = 2:1) with 5 replications; (2) 1.4U:1F (urine to faeces = 1.4:1) with 5 replications; and (3) 1U:1F (urine to faeces = 1:1) with 5 replications. Urine to faeces ratios (g N/g N) of 1.52 and 1.07, and 0.76 were used for 2U:1F, 1.4U:1F, and 1U:1F, respectively. The final manure volume of each incubation system, for all treatments, was 600 g.

Experimental settings

Ten-day laboratory experiments were conducted to incubate sheep manure to quantify NH_3 emissions, using an acid trap set up. In brief, the incubation system (adopted from Misselbrook *et al.* (2005)) consisted of an air pump (Aqua One 110, Stellar Ltd, China), an airflow meter (Darhor LZB-3WB 0.15–1.5 l/min, Hangzhou Darhor Technology Ltd, China), a water container (a 900-ml clip container; 12.5 cm high, 13.5 cm wide, 10.5 cm diameter, ANKO Ltd, China), a manure container (a 2.3-l clip container; 16.6 cm high; 16.4 cm diameter, ANKO Ltd, China), and an acid jar (Quickfit Flask Erlenmeyer 500-ml 29/30, Fisher Scientific Ltd, UK). The airflow meter adjusted flows to 1 l/min to maintain pressure, transfer moisture from the water container to the manure container and pass the manure gases into the acid trap. Daily prepared 0.5 M sulphuric acid (H_2SO_4; 500-ml) was used to capture the released NH_3.

The ambient temperature was measured by a laboratory thermometer 3 times/day at 10 am, 4 pm and 10 pm. All incubation systems were also checked for leakage at 10 am, 4 pm and 10 pm by placing an airflow meter before the acid jar for approximately one minute. Fifteen grams manure per incubation system was collected randomly from five different locations, using a straw, and the samples were stored in a freezer at −20°C. At the same time, 2 g manure samples were taken and mixed with 4 ml purified water (pH = 7) and shaken for 30 min prior to measuring pH (pH/ISE and EC/TDS Benchtop Meter-IC-HI5521-02, HANNA Ltd, Australia). Acid traps were replaced daily at 10 am and 15 ml of the acid solutions were sampled and stored in a freezer at −20°C.

Sample analyses

All manure samples were taken from the freezer and freeze-dried (Christ Freeze Dryer GAMMA 1-16 LSCplus, Christ Ltd, Germany) for 5 days. Then, they were ground through a 2-mm screen by tissuelyzer (QIAGEN Ltd, Germany) with a pulse frequency of 30 for 40 s. Manure samples (3 ± 0.5 mg) were weighed directly into tin capsules (pressed, standard weight 8 × 5 mm, Sercon Ltd., Gateway, UK) and analysed for N (g/kg) and ^{15}N (‰, ^{15}N comparative to total ^{14}N plus ^{15}N) on a 20–20 Europa isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, Cheshire, UK). On completion of the 10-day incubation periods, the analysis of acid trap samples was performed. Briefly, 15 ml of each acid sample was neutralized with 6 M sodium hydroxide (NaOH) to obtain a pH between 4.5 and 6. Then, NH_4^+-N concentrations of the acid samples were determined using a Segmented Flow Analyzer (SFA; San + +, Skalar, V 3.2). The limit of quantification was 0.2 mg/l and any values below this were not recorded. Values more than 20 mg/l were obtained using appropriate dilution and recalculation. All used chemicals in the current experiment were of analytical reagent grade, and all fresh acid solutions were prepared with distilled water. The composition of N isotope of manure mixture was expressed as $\delta^{15}N$ (‰) and calculated as:

$$\delta^{15}N = (R\ \text{sample} - R\ \text{standard})/R\ \text{standard},\ \text{where}$$

$$R = {}^{15}N/({}^{14}N + {}^{15}N)$$

The corrected $\delta^{15}N$ based on sample data at day zero ($\Delta^{15}N$; ‰) was also introduced as a possible biomarker in these experiments and expressed as $\Delta^{15}N$ using the formula:

$$\Delta^{15}N = \delta^{15}N_{\text{each day}} - \delta^{15}N_{\text{day zero}}$$

Statistical analyses

A one-way analysis of variance (ANOVA) was conducted to test for statistically significant differences among treatments. In experiment 1, the ANOVA with unequal sample sizes were used, as replication units were uneven. Fishers protected least significant difference (LSD) test were used to compare the mean values of treatments. The significant differences were set at $P < 0.05$ and trends were declared at $0.05 < P < 0.10$. As the incubation systems of each treatment were the replication units in these experiments, data per day from each treatment were analysed using repeated measurements, with treatment as treatment structure and replication as block. The statistical package of GenStat (version 16; VSN International Ltd., Hemel Hempstead, UK) was used for all statistical analyses.

Results

Experiment 1

The results from the first experiment are shown in Table 2. Manure-N (g/kg of DM) differed significantly among treatments ($P < 0.001$; Table 2). Manure in C and GM had approximately 21 and 24% higher N% than C + L, respectively ($P < 0.001$; Table 2). In addition, manure pH in C + L was lower than in C and GM ($P < 0.001$; Table 2). Manure temperature was approximately 3% higher in C + L and GM compared to C ($P = 0.006$; Table 2). Daily manure-N losses (g) varied among treatments (0.108, 0.028 and 0.090 g for C, C + L and GM, respectively; $P < 0.001$; Table 2). Added lignite in the C + L led to significant lower manure-N losses in comparison with C and GM groups ($P < 0.001$; Table 2). Moreover, cumulative manure-N loss

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Table 2. Manure composition, pH, temperature, nitrogen losses, ammonia emissions, and nitrogen isotopic discrimination in three treatments of Experiment 1; C, C + L and GM

	Treatment				
Items	C	C + L	GM	S.E.M.	*P* value
Manure DM, g/kg	114.8	251.0	151.0	–	–
Manure-N, g/kg of DM	24.7	20.4	25.3	0.12	< 0.001
Manure pH	8.9	8.2	8.9	0.02	< 0.001
Manure temperature, °C	17.5	18.0	18.0	0.09	0.006
Daily manure-N loss, g	0.108	0.028	0.090	0.0064	< 0.001
Cumulative manure-N loss, g/100 g	46.8	9.0	28.2	1.58	0.001
Daily emitted manure NH_3-N, g	0.093	0.013	0.078	0.0016	< 0.001
Cumulative emitted manure NH_3-N, g/100 g	86.6	56.5	88.3	8.63	0.020
Manure $\delta^{15}N_{(day\ zero)}$, ‰	1.95	10.15	2.95	0.255	< 0.001
Manure $\delta^{15}N_{(last\ day)}$, ‰	8.06	19.12	6.78	0.803	< 0.001
Manure $\Delta^{15}N_{(last\ day\ -\ day\ zero)}$, ‰	6.12	8.98	3.84	0.642	< 0.001

C, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg); C + L, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg) and mixed with 100 g lignite; GM, urine and faeces from sheep fed grape marc diet (control animal feed ration, 200 g/kg replaced with grape marc); S.E.M., Standard error of means; DM, dry matter; N, nitrogen; Cumulative manure-N loss: as g/100 g of manure-N; NH_3-N, nitrogen content in the form of ammonia; Cumulative emitted manure NH_3-N: as g/100 g of manure-N loss.
The presented numbers for manure DM, manure-N, manure pH, and manure temperature are based on the average over 10-day incubation period.

(as g/100 g of manure-N) was lower in C + L than C and GM by 81 and 68%, respectively ($P = 0.001$; Table 2). Daily emitted manure NH_3-N (g) was significantly different among treatments ($P < 0.001$; Table 2), with far less emitted manure NH_3-N from C + L (0.013 g) compared to C (0.093 g) and GM (0.078 g) treatments. Cumulative emitted manure NH_3-N (as g/100 g of manure-N loss) was lower in C + L than C and GM by 35 and 36%, respectively. ($P = 0.020$; Table 2). Manure $\delta^{15}N_{(day\ zero)}$ was highly significantly different among treatments ($P < 0.001$; Table 2) with far more enrichment in C + L (10.15‰) compared to C (1.95‰) and GM (2.95‰) treatments. Manure $\delta^{15}N_{(last\ day)}$ in C + L (19.12‰) was also significantly enriched than C (8.06‰) and GM (6.78‰) ($P < 0.001$; Table 2). Manure $\Delta^{15}N_{(last\ day\ -\ day\ zero)}$ was highly significantly different among treatments ($P < 0.001$; Table 2) with the highest enrichment in C + L (8.98‰) and the lowest in GM (3.84‰).

Cumulative emitted manure NH_3-N increased ($P < 0.001$) non-linearly ($R^2 = 0.99$) over the 10 days from 0.156 g, 0.010 and 0.151 g to 0.932, 0.132 and 0.691 g for C, C + L and GM treatments, respectively. Manure $\delta^{15}N$ also increased non-linearly during the incubation ($P < 0.001$; $R^2 = 0.96$) in 10 days from 3.9 and 3.4‰ to 8.1 and 6.8‰ for C and GM, respectively. However, a reduction in manure $\delta^{15}N$ occurred for C + L during the incubation from 20.5 to 19.1‰ ($R^2 = 0.48$; $P < 0.001$). The relationship between cumulative emitted manure NH_3-N and manure $\delta^{15}N$ ($P < 0.001$) was positive and strong for both C and GM ($R^2 = 0.96$ and $R^2 = 0.93$, respectively; Fig. 1a). However, the correlation between cumulative emitted manure NH_3-N and manure $\delta^{15}N$ was non-significant for C + L ($R^2 = 0.44$; $P = 0.128$; Fig. 1b). A combined equation for treatments C and GM ($Y = 0.0077\ X^2 + 0.1102\ X + 0.0645$) also showed that the relationship between cumulative manure emitted NH_3-N and manure $\delta^{15}N$ was positive and highly significant ($R^2 = 0.88$, S.E. = 0.120, $P < 0.001$). Moreover, a strong positive relationship was found between cumulative emitted manure NH_3-N and manure $\Delta^{15}N$ ($P < 0.001$) for C and GM ($R^2 = 0.96$ and $R^2 = 0.93$, respectively; Fig. 2a). A moderate negative, but significant, correlation ($R^2 = 0.44$; $P < 0.05$) between cumulative emitted manure NH_3-N and manure $\Delta^{15}N$ was found for C + L (Fig. 2b).

Experiment 2

The results from the second experiment are shown in Table 3. Total concentrations of manure-N (g/kg of DM) differed significantly between 2U:1F and 1.4U:1F ($P = 0.046$; Table 3). Manure pH in 2U:1F varied from 1U:1F ($P = 0.010$; Table 3). Manure pH in 2U:1F was significantly lower than 1U:1F ($P = 0.010$; Table 3). Manure temperature was approximately 6% higher in 1.4U:1F and 1U:1F compared to 2U:1F ($P = 0.008$; Table 3). Daily manure-N losses (g) were 0.098, 0.108 and 0.114 g for 2U:1F, 1.4U:1F and 1U:1F treatments, respectively ($P = 0.021$; Table 3). Moreover, cumulative manure-N losses (as g/100 g of manure-N) were significant among groups; 41.6, 39.7, and 36.9 for 2U:1F, 1.4U:1F and 1U:1F treatments, respectively ($P = 0.002$; Table 3). Daily emitted manure NH_3-N (g) was significantly higher in 2U:1F (0.097 g) compared to 1.4U:1F (0.090 g) and 1U:1F (0.089 g) ($P = 0.003$; Table 3). Cumulative emitted manure NH_3-N (as g/100 g of manure-N loss) was significantly higher in 2U:1F compared to 1.4U:1F and 1U:1F by 18 and 26%, respectively ($P < 0.001$; Table 3). Manure $\delta^{15}N_{(day\ zero)}$ was not significantly different among treatment ($P = 0.271$; Table 3). Manure $\delta^{15}N_{(last\ day)}$ was significantly more depleted in 1.4U:1F (9.33‰) compared to 2U:1F (9.88‰) and 1U:1F (9.71‰) ($P = 0.028$; Table 3). Manure $\Delta^{15}N_{(last\ day\ -\ day\ zero)}$ was not significantly different among treatments ($P = 0.133$; Table 3).

Cumulative emitted NH_3-N from manure increased non-linearly ($P < 0.001$; $R^2 = 0.99$) over the 10 days (0.828 g, 0.763 and 0.749 g for 2U:1F, 1.4U:1F, and 1U:1F treatments, respectively). Manure $\delta^{15}N$ also increased non-linearly ($P < 0.001$; $R^2 = 0.98$) over the 10 days (7.8, 6.8 and 7.0‰ for 2U:1F, 1.4U:1F and 1U:1F treatments, respectively). The relationship

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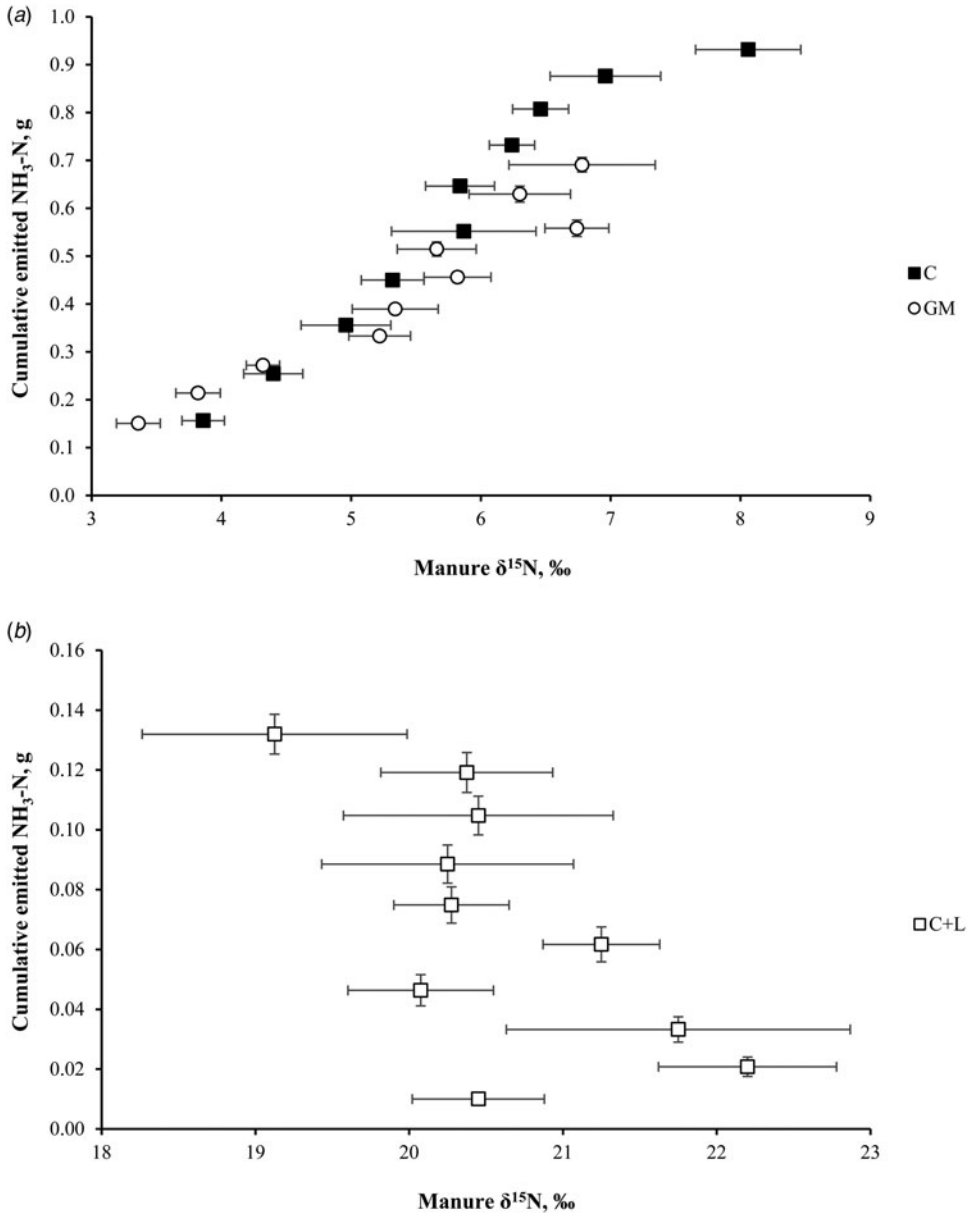

Figure 1. Relationship between cumulative emitted ammonia-nitrogen (NH_3-N) from manure and manure N isotopic discrimination ($\delta^{15}N$) during Experiment 1. (a) C, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg); GM, urine and faeces from sheep fed grape marc diet (control animal feed ration, 200 g/kg replaced with grape marc); (b) C + L, urine and faeces from sheep feed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg) and mixed with 100 g lignite. The error bars show standard error (S.E.).
Equations for Fig. 1 (a): C, Equation: $Y = -0.023\ X^2 + 0.4817\ X - 1.4103$, $R^2 = 0.96$, S.E. = 0.059, $P < 0.001$.
GM, Equation: $Y = 0.0123\ X^2 + 0.0205\ X - 0.0537$, $R^2 = 0.93$, S.E. = 0.055, $P < 0.001$.
Combined equation of C and GM: $Y = 0.0077\ X^2 + 0.1102\ X + 0.0645$, $R^2 = 0.88$, S.E. = 0.120, $P < 0.001$.
Equation for Fig. 1 (b): C + L, Equation: $Y = 0.0043\ X^2 - 0.2112\ X + 2.5772$, $R^2 = 0.44$, S.E. = 0.750, $P = 0.128$.

between cumulative manure emitted NH_3–N and manure $\delta^{15}N$ was positive and highly significant ($P < 0.001$) for all treatments (Fig. 3). A combined equation using results from all three treatments ($Y = 0.094e^{0.2193\ X}$) also showed that the relationship between cumulative manure emitted NH_3-N and manure $\delta^{15}N$ was positive and highly significant ($R^2 = 0.95$, S.E. = 0.066, $P < 0.001$). Additionally, positive relationships between cumulative manure emitted NH_3-N and manure $\Delta^{15}N$ were highly significant ($P < 0.001$) for all treatments ($R^2 = 0.99$, $R^2 = 0.95$; $R^2 = 0.95$, respectively for 2U:1F, 1.4U:1F and 1U:1F; Fig. 4).

Discussion

Manure composition effects on manure ammonia emissions and nitrogen losses

In Exp. 1, the application of lignite to manure reduced daily emitted NH_3-N (g) approximately 86% compared to C. This agrees with previous results. Chen *et al.* (2015) and Sun *et al.* (2016) showed that lignite application could reduce manure NH_3 emissions by 66 and 29.5% from beef cattle pens, respectively. Impraim *et al.* (2020) also observed that lignite-amended cattle manure retained 350 to 540 g/kg of N by avoiding NH_3 loss

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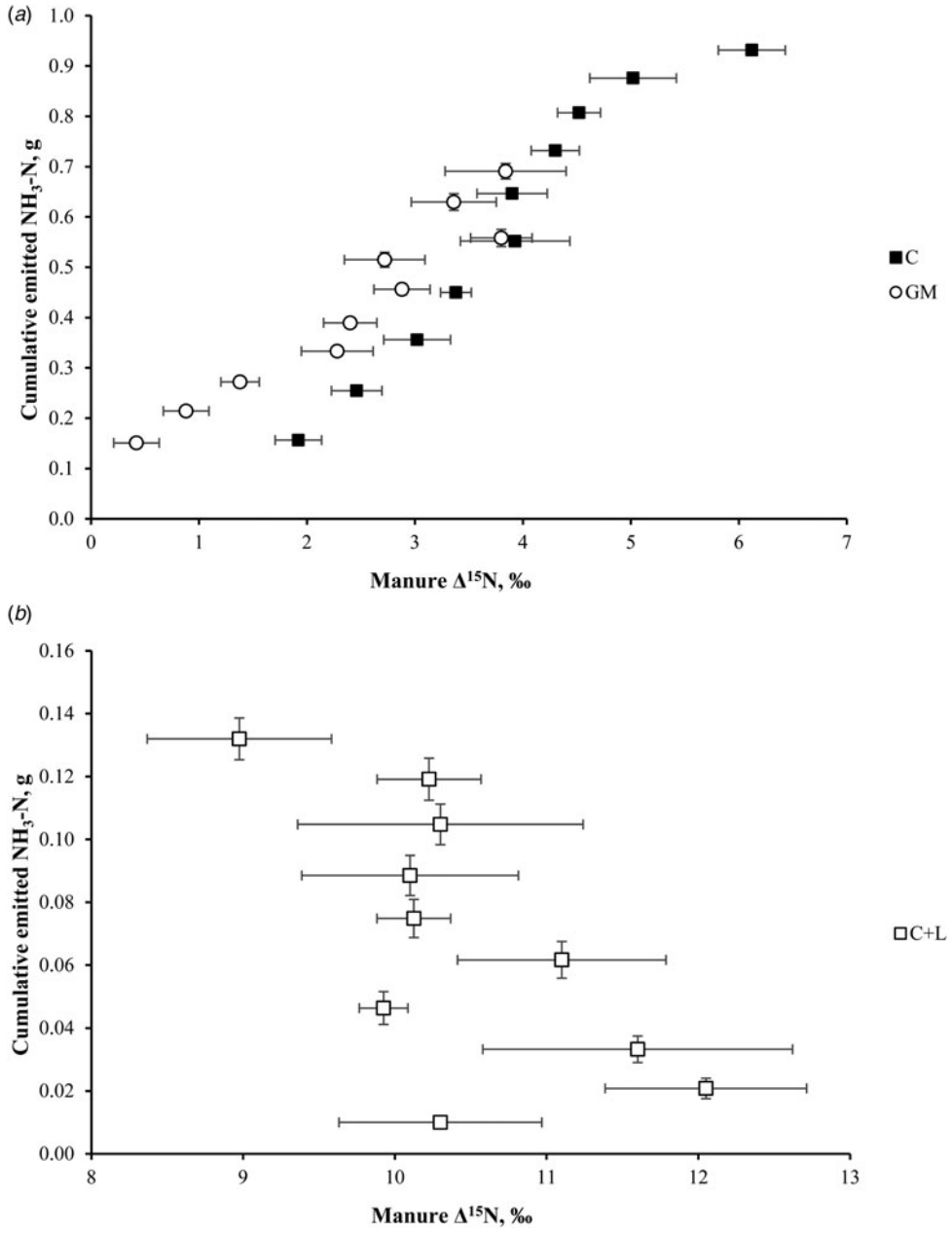

Figure 2. Relationship between cumulative emitted ammonia-nitrogen (NH_3-N) from manure and manure N isotopic discrimination corrected at day zero ($\Delta^{15}N$) during Experiment 1. (a) C, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg); GM, urine and faeces from sheep fed grape marc diet (control animal feed ration, 200 g/kg replaced with grape marc); (b) C + L, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg) and mixed with 100 g lignite. The error bars show standard error (s.e.).
Equations for Fig. 2 (a): C, Equation: $Y = -0.023\ X^2 + 0.3923\ X - 0.5624$, $R^2 = 0.96$, s.e. = 0.070, $P < 0.001$.
GM, Equation: $Y = 0.0123\ X^2 + 0.0927\ X + 0.1127$, $R^2 = 0.93$, s.e. = 0.053, $P < 0.001$.
Combined equation of C and GM: $Y = 0.0077\ X^2 + 0.1102\ X + 0.0645$, $R^2 = 0.88$, s.e. = 0.085, $P < 0.001$.
Equations for Fig. 2 (b): C + L, Equation: $Y = 0.0043\ X^2 - 0.1232\ X + 0.8805$, $R^2 = 0.44$, s.e. = 0.033, $P < 0.05$.

compared to manure that received no lignite application. Lignite's ability to mitigate NH_3 emissions from manure may be related to three major chemical characteristics: low pH (3.69), high CEC (96.8 cmol (+)/kg), and high labile carbon content (up to 200 g/kg) (Whitehead and Raistrick, 1993; McCrory and Hobbs, 2001; Chen *et al.*, 2015). The low pH and high buffering capacity of lignite alters the ratio of NH_4^+/NH_3 towards NH_4^+, which is non-volatile, leading to a reduction of NH_3 emissions. It has been proposed that the inclusion of GM into ruminant diets can also be useful for reducing manure NH_3 emissions as GM changes manure property (Lynch *et al.*, 2007). Tannin is present at high concentrations in GM (Spanghero *et al.*, 2009; Nudda *et al.*, 2015), and it has been shown that a ruminant diet supplemented with tannin from grape seed reduces NH_3 emissions (Waghorn *et al.*, 2002; Grainger *et al.*, 2009). However, Scuderi *et al.* (2019) observed that the inclusion of GM in a dairy cattle ration did not change N parameters (e.g., UN and FN). Our results showed that even though GM treatment had a higher

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Table 3. Manure composition, pH, temperature, nitrogen losses, and ammonia emissions in three different treatments of Experiment 2; 2U:1F, 1.4U:1F and 1U:1F

	Treatment				
Items	2U:1F	1.4U:1F	1U:1F	S.E.M.	*P* value
Manure DM, g/kg	93.9	117.3	140.8	–	–
Manure-N, g/kg of DM	23.7	23.3	23.5	0.13	0.046
Manure pH	9.23	9.36	9.47	0.059	0.010
Manure temperature, °C	8.5	9.0	9.0	0.03	0.008
Daily manure-N loss, g	0.098	0.108	0.114	0.0043	0.021
Cumulative manure-N loss, g/100 g	41.6	39.7	36.9	0.82	0.002
Daily emitted manure NH_3-N, g	0.097	0.090	0.089	0.0016	0.003
Cumulative emitted manure NH_3-N, g/100 g	98.3	83.2	78.3	3.16	< 0.001
Manure $\delta^{15}N_{(day\ zero)}$, ‰	0.41	1.05	1.29	0.520	0.271
Manure $\delta^{15}N_{(last\ day)}$, ‰	9.88	9.33	9.71	0.166	0.028
Manure $\Delta^{15}N_{(last\ day\ -\ day\ zero)}$, ‰	9.47	8.28	8.42	0.569	0.133

2U:1F, ratio of urine to faeces = 2:1; 1.4U:1F, ratio of urine to faeces = 1.4:1; 1U:1F, ratio of urine to faeces = 1:1; S.E.M., Standard error of means; DM, dry matter; N, nitrogen; Cumulative manure-N loss: as g/100 g of manure-N; NH_3-N, nitrogen content in the form of ammonia; Cumulative emitted manure NH_3-N: as g/100 g of manure-N loss.
The presented numbers for manure DM, manure-N, manure pH, and manure temperature are based on the average over 10-day incubation period.

manure-N content, a significant reduction in manure-N loss occurred without significant increase in manure NH_3-N emission.

In Exp. 2, the higher manure pH in 1U:1F in comparison with 2U:1F may have resulted in higher daily manure-N loss from 1U:1F (0.114 g *vs.* 0.098 g). The average ambient temperature during the experiment was 11°C. As Hristov *et al.* (2011) highlighted, estimating the influence of diets on potential gas-emission from manure depends on ambient temperature; however, as previously mentioned, *in vitro* methods have a limitation in that they do not account for the effects of environmental factors such as wind speed and turbulence over the manure surface. A significant lower cumulative manure-N loss (g/100 g) in 1U:1F compared to other two treatments can be partly explained by the lower proportion of urine to faeces volume. It is possible that manure-N could be emitted more in forms other than NH_3, such as through denitrification. This may relate to the lesser contribution of faeces than urine in the manure in 1.4U:1F and 1U:1F. It is generally accepted that manure NH_3 mainly derives from urea exposed to faecal urease (Wilkerson *et al.*, 1997). Thomsen (2000) investigated UN *v.* FN influences on ^{15}N in solid sheep manure during

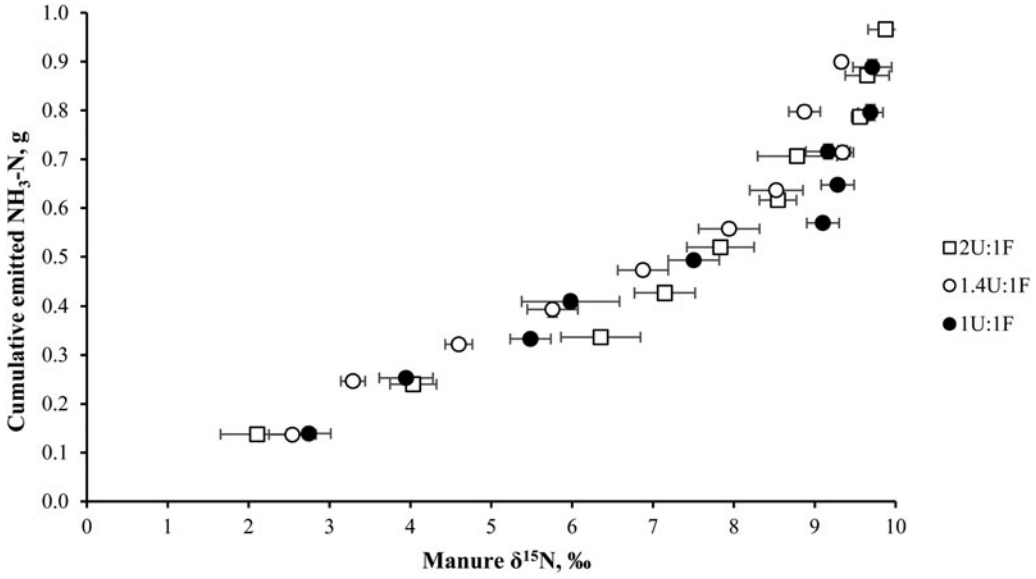

Figure 3. Relationship between cumulative emitted ammonia-nitrogen (NH_3-N) from manure and manure N isotopic discrimination ($\delta^{15}N$) during Experiment 2: 2U:1F, ratio of urine to faeces = 2:1; 1.4U:1F, ratio of urine to faeces = 1.4:1; 1U:1F, ratio of urine to faeces = 1:1. The error bars show standard error (S.E.).
2U:1F, Equation: $Y = 0.0823e^{0.2399\ X}$, $R^2 = 0.99$, S.E. = 0.043, $P < 0.001$.
1.4U:1F, Equation: $Y = 0.0999e^{0.2255\ X}$, $R^2 = 0.95$, S.E. = 0.068, $P < 0.001$.
1U:1F, Equation: $Y = 0.094e^{0.2193\ X}$, $R^2 = 0.95$, S.E. = 0.074, $P < 0.001$.
Combined equation of all three treatments: $Y = 0.094e^{0.2193\ X}$, $R^2 = 0.95$, S.E. = 0.066, $P < 0.001$.

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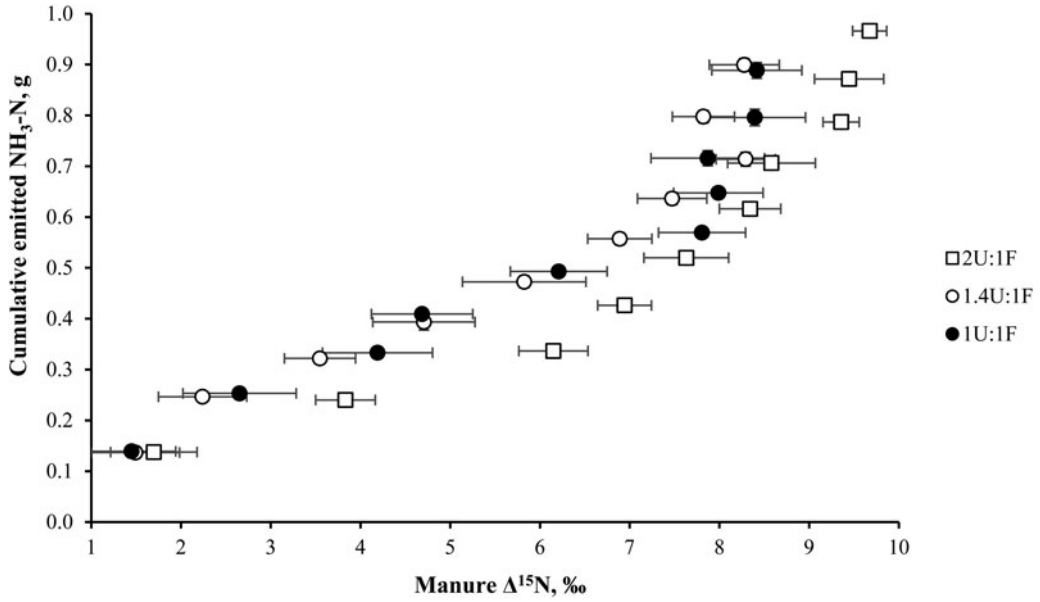

Figure 4. Relationship between cumulative emitted ammonia-nitrogen (NH_3-N) from manure and manure N isotopic discrimination corrected at day zero ($\Delta^{15}N$) during Experiment 2: 2U:1F, ratio of urine to faeces = 2:1; 1.4U:1F, ratio of urine to faeces = 1.4:1; 1U:1F, ratio of urine to faeces = 1:1. The error bars show standard error (S.E.).
2U:1F, Equation: $Y = 0.0896e^{0.2355\ X}$, $R^2 = 0.99$, S.E. = 0.103, $P < 0.001$.
1.4U:1F, Equation: $Y = 0.1266e^{0.2255\ X}$, $R^2 = 0.95$, S.E. = 0.073, $P < 0.001$.
1U:1F, Equation: $Y = 0.1248e^{0.2193\ X}$, $R^2 = 0.95$, S.E. = 0.077, $P < 0.001$.
Combined equation of all three treatments: $Y = 0.1216e^{0.2147\ X}$, $R^2 = 0.95$, S.E. = 0.090, $P < 0.001$.

both anaerobic and aerobic (composted) storage. In both situations, UN contributed most to total N losses. Lee *et al.* (2009) investigated UN *v.* FN effects on gaseous N emission from stored dairy cattle manure. The results demonstrated that in the first 10 days of manure storage, the main source of emitted NH_3 was from UN (i.e., 90 g/100 g). The same results were achieved in the study by Burchill *et al.* (2019) in cattle. The cumulative NH_3 emissions increased linearly with increasing urine N rate and emission factors.

A high cumulative manure-N loss (g/100 g) and cumulative NH_3-N emissions (g/100 g) occurred in Exp. 1 (except C + L) and Exp. 2. This result was due to the rapid increase in manure NH_3-N concentration during the 10-day incubation. Lee *et al.* (2011) mentioned that the rapid increase in NH_4^+ concentration in the manure was due to urinary-urea hydrolysis. In both experiments (except for manure mixture in C + L in the Exp. 1), the recapture of total N loss as NH_3-N from manure in the acid trap was high (~87 g/100 g and ~88 g/100 g for C and GM treatments, and ~98 g/100 g *vs.* ~83 g/100 g and ~78 g/100 g for 2U:1F, 1.4U:1F and 1U:1F, respectively). This suggests that the acid trap captured NH_3-N emitted from manure effectively. This result is in contrast with the result by Ndegwa *et al.* (2008), who reported that the efficiency of NH_3-N trapping was reduced with an increase in emitted NH_3-N. The scenario is different for the manure mixture in C + L. The effectiveness of the recapture of NH_3-N from the manure mixture in the acid trap was only moderate (~57 g/100 g), which could simply be the sensitivity of the NH_3-N analysis, as there were much lower losses with this treatment. It seems likely that nitrous oxide emissions may also have occurred for manure C + L, due to nitrification and denitrification. Earlier reports (Bussink and Oenema, 1998; Harper *et al.*, 2000) showed that reduction of nitrate to N_2O and dinitrogen gas (N_2) might be significant sources of N loss from lagoons/retention pond. Jones *et al.* (2000) showed that several chemical and biological mechanisms might exist for N_2 formation during the storage of manure.

Manure ammonia-nitrogen and nitrogen isotopic discrimination changes over experiment period

In Exp. 1, despite an increase of N in the manure mixture in C + L compared to manure-N in C (2.98 g *vs.* 2.35 g, respectively), the cumulative emitted manure NH_3-N in C + L was approximately 6 times less than C, which might be due to a lower proportion of water in the C + L (i.e., higher manure DM [g/kg]), and lignite lowering the pH. This result showed the effectiveness of lignite to reduce manure NH_3-N despite more N being present in the C + L mixture. Despite the increase of manure-N in GM compared to C (2.80 g *vs.* 2.35 g respectively; Table 3), and the manure content being 72 g higher in GM compared to C, the cumulative manure NH_3-N was approximately 44% less than C during 10-day incubation. As UN/FN and the manure-N concentration in GM were less than C (~40%), this might be a reason for the lower cumulative emitted NH_3-N from GM compared to C manure. Another possible reason for the reduced cumulative emitted NH_3-N from GM manure might be because of the reduced ratio of urine to faeces in GM than C. In Exp. 2, the highest cumulative emitted NH_3-N for 2U:1F manure and the lowest cumulative emitted NH_3-N from 1U:1F manure were more likely due to the highest and lowest proportion of urea to NH_3 content of the manure in 2U:1F and 1U:1F manure, respectively, compared to the other treatments. As Tamminga (1996) and Ndegwa *et al.* (2008) demonstrated, urine to faeces ratio is one of the major factors that influences manure NH_3 emissions.

The results from Exp. 2 suggested that the emitted manure NH_3-N might depend partly on manure-N content and partly

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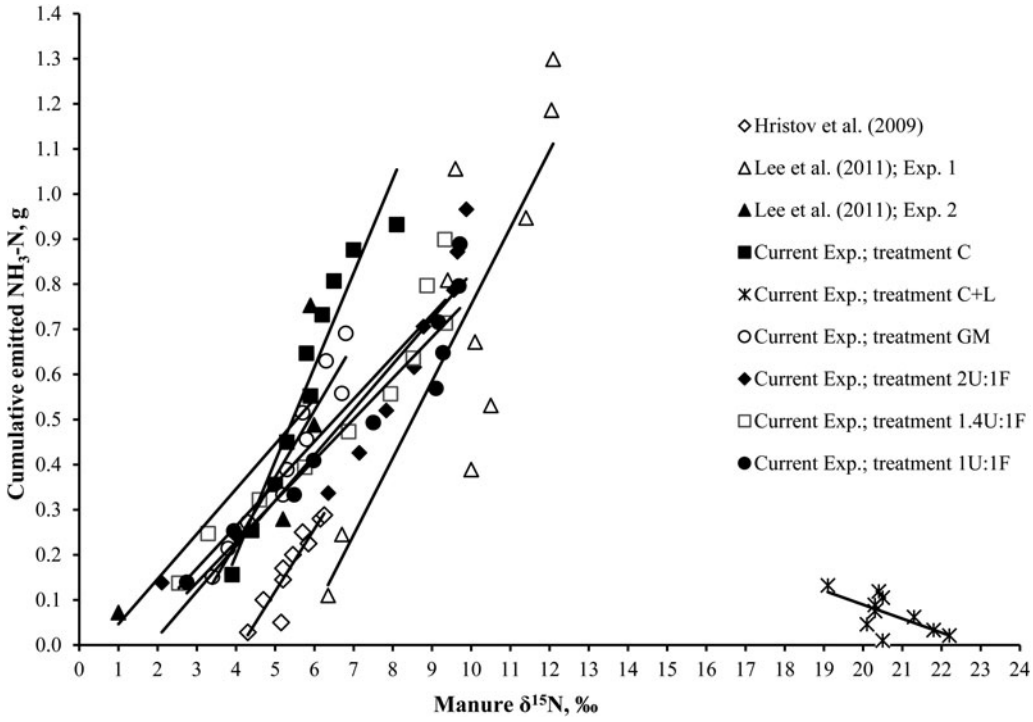

Figure 5. Relationship between cumulative emitted ammonia-nitrogen (NH_3-N) from manure and manure N isotopic discrimination ($\delta^{15}N$) during experiments : Hristov *et al*., 2009 (in cattle); Lee *et al*., 2011 (in cattle); Current experiment (Experiment 1: in sheep): C, urine and faeces from sheep fed control diet (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg); C + L, urine and faeces from sheep fed control diet control (550 g lucerne hay/kg, 400 g barley grain/kg, and 50 g bean/kg) and mixed with 100 g lignite; GM, urine and faeces from sheep fed grape marc diet (control animal feed ration, 200 g/kg replaced with grape marc); Current experiment (Experiment 2: in sheep): 2U:1F, ratio of urine to faeces = 2:1; 1.4U:1F, ratio of urine to faeces = 1.4:1; 1U:1F, ratio of urine to faeces = 1:1.

on bacterial enzymes in the manure. In general, the levels of NH_3-N emitted in both experiments (except for C + L group) were in the broad range compared to Hristov *et al.* (2009) and within the range identified by Lee *et al.* (2011) in large ruminant experiments. All relationships have a similar slope (i.e., Fig. 5), suggesting that for a given amount of NH_3-N release, there was a similar amount of discrimination of N isotopes. The differences in starting values of $\delta^{15}N$ among experiments highlighted the point that part of the discrimination of N isotopes may derive from other N losses than NH_3. For instance, the loss of N_2O or N_2 from denitrification, which would also lead to discrimination (Bussink and Oenema, 1998; Harper *et al.*, 2000). This is likely reflected in C + L of Exp. 1, which had much less NH_3-N loss. In both experiments, an increase in manure $\delta^{15}N$, was similar to the results described by Hristov *et al.* (2009) and Lee *et al.* (2011). However, in the Exp. 1, manure $\delta^{15}N$ for C + L increased from 20.5 to 22.2‰ (day 1 to day 2) and then decreased from 22.2 to 19.1‰. We do not have an explanation for this observation. However, it may be related to (1) some reactions between lignite and manure, causing the discrimination of N isotopes; (2) N loss in gases other than NH_3 in C + L; and/or (3) other N losses involving the discrimination of N isotopes.

Manure ammonia-nitrogen emissions in relationship with manure nitrogen isotopic discrimination

Our experiments mainly aimed to test the relationship between $\delta^{15}N$ of manure and cumulative emitted manure NH_3-N. The values of cumulative emitted NH_3-N and manure $\delta^{15}N$ relationship for treatments C and GM in Exp. 1 and all treatments in Exp. 2 were in the upper range shown by Hristov *et al.* (2009) and in the range described by Lee *et al.* (2011). A strongly positive relationship between cumulative emitted NH_3-N and manure $\delta^{15}N$ in both experiments (except for C + L) is consistent with the results of other reports in large ruminants (Hristov *et al.*, 2006; Hristov *et al.*, 2009; Lee *et al.*, 2011). Excluding results from the C + L treatment, an increase in manure $\delta^{15}N$ coincided with an increase in cumulative NH_3-N emissions. Lee *et al.* (2011) indicated that the rapid increase in $\delta^{15}N$ might be due to loss of depleted ^{15}N of NH_3-N, due to urea hydrolysis to NH_4^+. Therefore, the NH_3-N emissions and the discrimination of N isotopes could directly link. However, the ^{15}N measurement of NH_3-N was not investigated in these experiments. In Lee *et al.* (2011) this rapid increase in $\delta^{15}N$ happened at the beginning of the manure incubation. Lignite $\delta^{15}N$ measurement (i.e., for C + L) was highly variable in the current experiment, but less variation was observed in N (g/kg) for lignite; therefore, caution is needed when measuring $\delta^{15}N$ from lignite.

Conclusion

These two laboratory experiments confirmed that manure-N content and manure properties are major factors determining manure NH_3 emissions. The use of lignite application in sheep manure and the inclusion of GM into sheep diets can significantly reduce manure NH_3 emissions. A non-linear positive relationship between $\delta^{15}N$ of manure and NH_3 emissions was observed during 10-day incubations of manure in both experiments, except for manure treated with lignite. These experiments confirmed previous reports that manure $\delta^{15}N$ and $\Delta^{15}N$ may be valuable biomarkers for estimating NH_3 emissions from sheep manure.

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Authors' contributions. Khanaki, H.: Conceptualization, Investigation, Methodology, Sampling, Formal analysis, Writing – Original Draft and Revising; Dewhurst, R.J.: Supervision, Formal analysis, Methodology (supporting), Editing and Finalizing manuscript for submission; Leury B.J.: Supervision, Methodology (supporting), Editing and Finalizing manuscript for submission; Song, Y.: Conceptualization, Methodology, Formal analysis and Editing; Chen, D.; Editing and Finalizing manuscript for submission; Cheng, L.: Supervision, Conceptualization, Methodology, Formal analysis, Writing – Original Draft and Revising.

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Competing interests. None.

Ethical standards. The animals used in the study were handled according to the University of Melbourne Animal Ethics Committee, with experimental procedures approved by the University of Melbourne Animal Care Committee.

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